

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>6072.204-WO</b>	<b>FOR FURTHER ACTION</b>	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. <b>PCT/DK 01/ 00020</b>	International filing date (day/month/year) <b>12/01/2001</b>	(Earliest) Priority Date (day/month/year) <b>12/01/2000</b>
Applicant <b>NOVOZYMES A/S et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of **5** sheets.

It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
    - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
  - b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
    - contained in the international application in written form.
    - filed together with the international application in computer readable form.
    - furnished subsequently to this Authority in written form.
    - furnished subsequently to this Authority in computer readable form.
    - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
    - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
2.  Certain claims were found unsearchable (See Box I).
3.  Unity of invention is lacking (see Box II).

4. With regard to the **title**,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 01/00020

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-22

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-22

A method of producing a variant of a parent pullulanase

2. Claims: 23,28,33-37,40 and part of 31,32,39,41-47 and 59

A pullulanase variant having improved thermostability compared to the Patent pullulanase

3. Claims: 24,29,38 and part of claims 31,32,41-47 and 59

A pullulanase variant having increased isoamylase activity

4. Claims: 25,30 and part of claims 31,32,39,41,42, 44-47 and 59

A pullulanase variant having altered pH dependent activity compared to the parent pullulanase

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 01/00020

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 C12N9/44 C12P19/16

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE STN INTERNATIONAL [Online]            CAPLUS, CAPLUS accession no. 1999:702733;            document no. 132:31329,            YAMASHITA, MITSUO: "Monograph: Studies on            the improvement of functions useful            proteins"            XP002901781            abstract            &amp; SEIBUTSU KOGAKU KAISHA,            vol. 77, no. 8, 1999, pages 345-357,            ---            -/-</p>	1-22

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 June 2001

Date of mailing of the international search report

01.02.2002

Name and mailing address of the ISA

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 Fax: (+31-70) 340-3016

Authorized officer

Yvonne Siösteen

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 01/00020

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A	DATABASE STN INTERNATIONAL [Online] Medline, Medline accession no. 9879259; KATSUYA Y ET AL: "Three-dimensional structure of pseudomonas isoamylase at 2.2 Å resolution" XP002901784 abstract & JOURNAL OF MOLECULAR BIOLOGY, 4 September 1998 (1998-09-04), pages 885-897, Englang ---	1-22
A	WO 98 38287 A (NOVO NORDISK AS) 3 September 1998 (1998-09-03) the whole document ---	1-22
A	WO 99 01544 A (NOVO NORDISK AS) 14 January 1999 (1999-01-14) the whole document -----	1-22

## PATENT COOPERATION TREATY

## PCT

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EPO-Internal

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 June 2001

Date of mailing of the international search report

01.02.2002

Name and mailing address of the ISA

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Authorized officer

Yvonne Siösteen

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 01/00020

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A	WO 99 01544 A (NOVO NORDISK AS) 14 January 1999 (1999-01-14) the whole document -----	1-22

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/DK 01/00020

Patent document cited in search report		Publication date	Patent family member(s)	Publication date	
US 4560651	A	24-12-1985	AT 28216 T AU 548122 B2 AU 8280882 A BE 892904 A1 CA 1199292 A1 CH 659825 A5 DE 3276707 D1 DK 172882 A ,B, EP 0063909 A1 ES 511504 D0 ES 8307291 A1 ES 520561 D0 ES 8500994 A1 FI 821355 A ,B, FR 2504148 A1 GB 2097405 A ,B IE 53031 B1 IN 155660 A1 IN 160657 A1 IT 1190780 B JP 1491638 C JP 57174089 A JP 62025036 B JP 61043994 A KR 8902413 B1 MX 174149 B MX 7428 E		15-07-1987 21-11-1985 28-10-1982 19-10-1982 14-01-1986 27-02-1987 13-08-1987 29-12-1982 03-11-1982 16-06-1983 16-10-1983 01-11-1984 01-02-1985 21-10-1982 22-10-1982 03-11-1982 11-05-1988 16-02-1985 25-07-1987 24-02-1988 07-04-1989 26-10-1982 01-06-1987 03-03-1986 03-07-1989 25-04-1994 28-10-1988
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DIALOG(R) File 155: MEDLINE

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09879259 98387895 PMID: 9719642

Three-dimensional structure of Pseudomonas isoamylase at 2.2 Å resolution.

Katsuya Y; Mezaki Y; Kubota M; Matsuura Y  
Hyogo Prefectural Institute of Industrial Research, Suma-ku, Kobe,  
654-0037, Japan. [ykatsuya@hyogo-kp.go.jp](mailto:ykatsuya@hyogo-kp.go.jp)  
Journal of molecular biology (ENGLAND) Sep 4 1998, 281 (5) p885-97,  
ISSN 0022-2836 Journal Code: J6V

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The three-dimensional structure of isoamylase from *Pseudomonas amyloferamosa*, which hydrolyzes alpha-1,6-glucosidic linkages of amylopectin and glycogen, has been determined by X-ray structure analysis. The enzyme has 750 amino acid residues and a molecular mass of 80 kDa, and it can be crystallized from ammonium sulfate solution. The structure was elucidated by the multiple isomorphous replacement method and refined at 2.2 Å resolution, resulting in a final R-factor of 0.161 for significant reflections with a root-mean-square deviation from ideality in bond lengths of 0.009 Å. The analysis revealed that in the N-terminal region, isoamylase has a novel extra domain that we call domain N, whose three-dimensional structure has not so far been reported. It has a (beta/alpha)8-barrel-type supersecondary structure in the catalytic domain common to the alpha-amylase family enzymes, though the barrel is incomplete, with a deletion of an alpha-helix between the fifth and sixth beta-strands. A long excused region is present between the third beta-strand and the third alpha-helix of the barrel but, in contrast to the so-called domain B that has been identified in the other enzymes of alpha-amylase family, it cannot be considered to be an independent domain, because this loop forms a globular cluster together with the loop between the fourth beta-strand and the fourth alpha-helix. Isoamylase contains a bound calcium ion, but this is not in the same position as the conserved calcium ion that has been reported in other alpha-amylase family enzymes. Copyright 1998 Academic Press

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